Perspectives in Metabolic Flux Mapping

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Metabolic flux maps provide a quantitative depiction of carbon flow through competing metabolic pathways, thus providing: analysis of substrate utilization and product formation; flexibility or rigidity of carbon flow at network nodes; the rate of a given enzymatic reaction in vivo; and inferred availability of NADPH or ATP. Thus, metabolic fluxes are an important physiological characteristic complementary to levels of transcripts, proteins, and metabolites. The system-wide quantification of intracellular fluxes in an organism is called metabolic flux analysis (MFA). The most basic approach to MFA is stoichiometric MFA, which involves writing balances for intracellular metabolites based on the stoichiometry of the biochemical reactions in the metabolic network. This results in a system of linear equations, which are solved by employing extracellular and biomass synthesis flux measurements to resolve some or all degrees of freedom. Genome-wide or in silico flux models provide the solution space of feasible fluxes resulting from optimization of the balances to a global cellular goal, such as maximum growth rate. Recently, constraints to the in silico models provided by data from ^{13}C labeling experiments, have narrowed the solution space.

^{13}C metabolic flux analysis (^{13}C MFA), with isotope detection via GC/MS or NMR of metabolites (e.g. amino acids from hydrolyzed protein), quantifies intracellular metabolic fluxes for smaller reaction networks, where the fluxes are completely determined (in constrast to the in silico models). ^{13}C MFA provides redundant measurements for flux quantification, as well as testing the consistency of the network topology for the physiological conditions. Isotopomers, which are isomers of a metabolite that differ in the labeling state (^{13}C or ^{12}C) of their individual carbon atoms, are a central concept in the analysis and mathematical modeling of ^{13}C MFA.

Increasing levels of information can be obtained from ^{13}C labeling data when coupled with a stoichiometric model of the biochemical pathways and computational methods to solve for flux data in the smaller network. More rigorous analysis is indicated as one moves from analytical (a few flux ratios at metabolic branchpoints) to ^{13}C constrained flux analysis (stoichiometric model with a few flux ratios as constraints) to fully integrated determination of fluxes from all the experimental data and the stoichiometric and isotopomer balances. Iterative methods have been used to solve the full relationship of isotopomer balances and the NMR or GC measurements to provide consistency, and routines to minimize error from the overdetermined data sets are required.

^{13}C MFA studies of aerobic glycolysis in microorganisms have become “higher throughput” since simplifications to the metabolic network can be made, and ^{13}C constrained flux analysis can be used. For alternative physiological conditions, for example where anapleurotic pathways are active, reversibility of reactions are indicated, or substrates other than glucose are used, the development of a consistent network

topology and the strategy for the choice of the label to obtain identifiable fluxes are not as straight-forward. Furthermore, due to compartmentation and the existence of parallel pathways in plants, more experimental measurements are needed than in microbial systems, and the number of isotopomer balances increases, further increasing the computational burden. As a note, to date in silico models for plants have not been developed. Thus, at this point, these more challenging systems are not yet ready for "high-throughput" measurements. However, a growing knowledge base in ^{13}C MFA in these systems should enable movement towards more genome-wide flux estimation. This presentation will summarize these points with approaches from our laboratory in determining ^{13}C-based metabolic flux maps in plants and microbes.